Exhibit 1

Fluorouracil Plus Racemic Leucovorin Versus Fluorouracil Combined With the Pure *l*-Isomer of Leucovorin for the Treatment of Advanced Colorectal Cancer: A Randomized Phase III Study

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Purpose: To compare the efficacy and toxicity of fluorouracil (FU) and racemic leucovorin (*d,l*-LV) versus FU combined with the *l*-isomer of leucovorin (*l*-LV) in the treatment of advanced colorectal cancer.

Patients and Methods: A total of 248 patients with advanced measurable colorectal cancer previously unexposed to chemotherapy were randomly assigned to treatment with either FU (400 mg/m^2/d by intravenous [IV] infusion for 2 hours) and racemic LV (100 mg/m^2/d by IV bolus injection) given for 5 consecutive days, or the combination of FU and the pure *l*-isomer of LV using the same dose schedule. In both treatment arms, courses were administered every 28 days if toxicity allowed for a total of 6 months, unless evidence of tumor progression was documented earlier.

Results: There were no significant differences between the FU/racemic LV and the FU/*l*-LV arm in the overall response rate (25% *v* 32%), duration of response (7.2 *v* 8.0 months), median time to progression or death (6.25 *v* 8.0 months), or median overall survival time (14.5 *v* 15.0 months). Except for minor myeloid toxic effects associated with FU/*l*-LV, there was also no significant difference in terms of adverse reactions. Gastrointestinal symptoms, specifically mucositis and diarrhea, were less frequent and less severe in both treatment arms compared with other trials with FU/racemic LV reported in the literature, which might be because of the prolonged administration of FU used in both arms.

Conclusion: The combination of FU/*l*-LV produced response rates, response durations, and survival times similar to those with FU/*d,l*-LV. Biochemical modulation of FU by either pure *l*-LV or racemic LV thus appears to result in equivalent clinical efficacy.

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COLORECTAL adenocarcinoma is one of the most common solid tumors in the Western societies, and more than 40% of patients will have locoregional recurrences and/or distant metastases at some point during the course of their disease.[1] The mainstay of palliative treatment for advanced colorectal cancer is cytotoxic chemotherapy, which has been shown to prolong survival and improve quality of life in randomized studies.[2,3] The fluorinated pyrimidines, fluorouracil (FU), and its deoxyribose floxuridine are the most active agents, but they have been shown to be more effective when given with biochemical modulators such as leucovorin.[4] However, there is considerable room for improvement in terms of the response rates and tolerability of chemotherapy, and the optimum regimen has yet to be determined.[5,6]

Modulation of FU by leucovorin (5-formyltetrahydrofolate or folinic acid) is caused by the interaction of thymidylate synthase, 5-fluoro-2′-deoxyuridine monophosphate, and 5,10-methylenetetrahydrofolate, which leads to the formation of a stable ternary complex with concomitant enzyme inactivation.[7] The formulation of leucovorin used in preclinical and clinical studies consists of a mixture of equal parts of two diastereomers differing in chirality at the C-6 carbon of the pteridine ring. Only the levorotatory isomer of leucovorin (*l*-LV) is transformed into active folate cofactors. The unnatural isomer (*d*-LV), however, is not inert; investigators have shown that it competes with the active (*l*-) form for uptake by cells.[8,9] It has also been reported that it is an inhibitor and a substrate for folypolyglutamate synthetase,[10] an enzyme that has a critical role in the folate-induced modulation of the fluoropyrimidines.[11] Because after intravenous (IV) administration of racemic LV the *d*-form accumulates in plasma at concentrations highly exceeding those of the *l*-form,[12] the possibility of a deleterious effect of the unnatural isomer on the modulation of FU cannot be ruled out.[8-10] Though tissue culture experiments have suggested that this phenomenon may only be significant with use of extremely high LV concentrations,[13] a recent analysis

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of human tumor biopsies obtained 30 minutes after IV administration of 200 mg/m^2 racemic LV demonstrated that *d*-LV may in fact partially inhibit tissue distribution of the effective *l*-form.[14]

In agreement with pharmacokinetic and in vitro studies of *l*-LV, showing similar effects at half doses,[15] and improved tissue uptake in liver metastases from gastrointestinal malignancies[14] when compared with racemic LV, impressive response rates have been reported in early phase I/II studies in colorectal cancer patients treated with FU plus *l*-LV.[16] However, any advantage over racemic LV must be confirmed in a randomized trial. We report here the results of such a controlled trial in previously untreated patients with advanced colorectal adenocarcinoma. Because previous studies comparing various (ie, low-dose *v* high-dose weekly as well as 5-day bolus) racemic LV/FU regimens have failed to demonstrate a direct relationship between the dose of the biochemical modulator and the therapeutic effectiveness,[17-19] and because the costs of the pure *l*-isomer are substantially higher than that of racemic LV (approximately 2.2-fold in Austria), we decided to compare identical rather than equipotent doses of the two LV preparations. In fact, we wished to determine if use of a double-the-effective dose of LV in combination with FU (*l*-LV arm) would result in any substantial/clinically relevant difference in therapeutic effectiveness or incidence of adverse reactions. The dose and schedule of FU, specifically the extended duration of the infusion used in both treatment arms, was derived from previous clinical studies, suggesting less frequent and severe treatment-associated toxicities.[16,20]

PATIENTS AND METHODS

Eligibility Criteria

Patients were required to have histologically confirmed metastatic and/or recurrent colorectal adenocarcinoma that was not amenable to curative surgical resection. Each patient had to have measurable disease that could be assessed by radiographic measurements, and patients were only eligible when a progression of measurable tumor of at least 25% in size or the appearance of new metastases had been demonstrated within the last 8 weeks. Further eligibility criteria included a World Health Organization (WHO) performance status of 0 to 3, a life expectancy of at least 3 months, and adequate bone marrow (WBC count > 3,500/μL, platelet count > 100,000/μL), renal (serum creatinine concentration < 132 μmol), and hepatic (serum bilirubin level < 34 μmol/L, serum transaminase level < 100 IU/L) function. All patients were previously untreated by chemotherapy, and any kind of radiotherapy must have been stopped at least 8 weeks before study entry. Patients were excluded if they had CNS metastases, presence of osseous metastases as the sole tumor site, serious and/or uncontrolled concurrent medical illness, or a history of other malignancies unless basal carcinoma of the skin or carcinoma in situ of the cervix that was treated adequately. Informed consent was obtained from all patients before randomization, and the study was approved by the Ethical Committee of the University of Vienna.

Randomization Procedures

The participating centers entered patients by telephone at the central study office located at the University of Vienna. After confirming eligibility in agreement with the protocol, patients were stratified according to WHO performance status (score 0 to 1 *v* 2 to 3), presence or absence of liver metastases, and weight loss in the 6 months before study entry. Patients were then randomly assigned to one treatment regimen by the central office. Balance across strata was attained using the method reported by Pocock and Simon.[21]

Treatment Protocol

Patients in both treatment arms received identical dose regimens of FU combined with either racemic LV (Calciumfolinat, Ebewe-Arzneimittel Ges.m.b.H., Unterach, Austria) or *l*-LV (L-Leukovorin, Lederle Arzneimittel-Cyanamid Ges.m.b.H., Vienna, Austria). Racemic LV or *l*-LV was administered at 100 mg/m^2/d by IV bolus injection immediately followed by FU 400 mg/m^2/d administered as a 2-hour infusion.

Chemotherapeutic drugs were given on 5 consecutive days at 4-week intervals for a total of 6 months or until there was evidence of tumor progression. In case of relapse in patients who achieved objective response or stable disease, original therapy was reinstituted for a maximum duration of another six treatment cycles. Treatments were delayed weekly if patients had not recovered from toxicity. In case of WHO grade 3 or 4 toxicity, the FU dosage was reduced by 20% on subsequent courses.

Pretreatment evaluation included a complete medical history, physical examination, routine hematology and biochemistry analysis, chest x-ray, ECG, and computed tomographic (CT) scan of the chest, abdomen, and pelvis.

Toxicity and Response Criteria

Toxicity was evaluated according to WHO criteria.[22] Hematologic parameters were assessed weekly, and all other adverse reactions were evaluated retrospectively before the next cycle. Tumor size was measured by CT scan, x-ray, or any other technique that allows retrospective and independent reassessment every 8 weeks. Objective responses had to be confirmed in one subsequent examination after a 4-week interval. Centers were required to be consistent with respect to the method of assessment for each patient. A complete response (CR) was defined as the disappearance of all detectable disease on two consecutive evaluations 4 weeks apart. A partial response (PR) was defined as a greater than 50% reduction of the summed products of the two greatest diameters of all measurable disease, with no new lesions appearing and none progressing for at least 4 consecutive weeks. No change was defined as a less than 50% reduction and less than 25% increase of measurable tumor lesions lasting for at least 8 weeks. Patients were considered to have progressive disease (PD) if the measurable tumor lesions increased by greater than 25% according to initial staging or if new lesions appeared within the first 2 months of therapy. All CR and PR decisions were reviewed by an independent external panel of oncologists and radiologists blinded to treatment received. Survival was determined from the date of first treatment until death or until the patient

was last examined alive. Time to progression was determined as the interval between the date of first treatment and the date PD was first observed.

Statistical Considerations

The study was designed to detect an improvement in patient survival from 30% to 45% at 1 year, with 120 patients per arm providing at least 90% power (α = 5%). Further end points were objective response, progression-free survival, and toxicity. Pretreatment characteristics, tumor response rates, and treatment toxicities in the two arms were compared using the χ^2 test. Patient survival and progression-free survival were examined with the Kaplan-Meier product-limit method,[23] and treatment arms were compared with the log-rank test.[24]

RESULTS

Between October 1991 and December 1994, a total of 248 patients from eight centers were accrued to the study. Of these patients, 125 were randomized to receive FU/racemic LV and 123 to FU/*l*-LV. Seven patients did not receive the allocated treatment subsequent to randomization because they were found to be ineligible for the following reasons: lack of histologic confirmation, metastatic disease not confirmed on CT (n = 2), noncolonic primary tumor, previous FU treatment (n = 2), and uncontrolled thrombembolism. Thus 241 of 248 patients (92.2%) are included in this analysis. The two groups were well matched for pretreatment characteristics, as listed in Table 1. The median age was 65 years, and the male-to-female ratio was 3:2. The large majority of patients had a WHO performance status of 0 or 1, two thirds had no or only minimal weight loss within 6 months before study entry, and most had moderately differentiated tumors metastatic to the liver. The primary tumor site was colonic in 151 patients and rectal/rectosigmoid in 90. Five patients had been previously treated with radiotherapy, four of whom had rectal primary tumors.

Tumor Response

Response to treatment according to the study arm is listed in Table 2. The overall objective response rates of 25% (FU/racemic LV) and 32% (FU/*l*-LV) were not significantly different (P = .25). Similarly, both median time to response (defined as the time from the start of treatment to the first record of the patient's best response) of 2.7 versus 2.4 months (P = .52) and median durations of overall responses (CRs and PRs) of 7.2 versus 8.0 months (P = .65) suggested only a marginal advantage in favor of the patients treated with FU/*l*-LV. The frequencies of responses by sites of disease did not differ appreciably between the two treatment regimens.

Table 1. Pretreatment Characteristics

Characteristic	FU/LV	FU/*l*-LV	Total
No. of patients			
Entered	125	123	248
Assessable	122	119	241
Sex			
Male	73	66	139
Female	49	53	102
Age (years)			
Median	65	64	65
Range	22-75	26-75	22-75
WHO performance status			
0	45	35	80
1	48	55	103
2	15	17	32
3	14	12	26
Weight loss before study (kg)			
≤ 5	81	79	160
> 5	41	40	81
Location of primary tumor			
Colon	75	76	151
Rectum	47	43	90
Disease-free interval (months)			
< 6	65	60	125
6-12	21	26	47
> 12	36	33	69
Location of metastases			
Liver	78	76	154
Lung	26	28	54
Abdominopelvic mass	51	54	105
Other*	3	5	8
No. of metastatic sites			
Single	87	81	168
Multiple	35	38	73
Histologic grading			
G1	9	8	17
G2	85	85	170
G3	20	15	35
Gx	8	11	19
Number of treatment courses			
Median	6	6	6
Range	1-12	1-13	1-13

*Includes bone, mediastinum, and lymph nodes.

Table 2. Overall Tumor Response

	FU/LV (n = 122)		FU/*l*-LV (n = 119)	
Tumor Response	No.	%	No.	%
CR	4	3	6	5
PR	26	21	32	27
No change	57	47	51	43
PD	27	22	17	14
Not assessable*	8	7	13	11
Overall response	30	25	38	32

*Inadequate follow-up data available to evaluate tumor response.

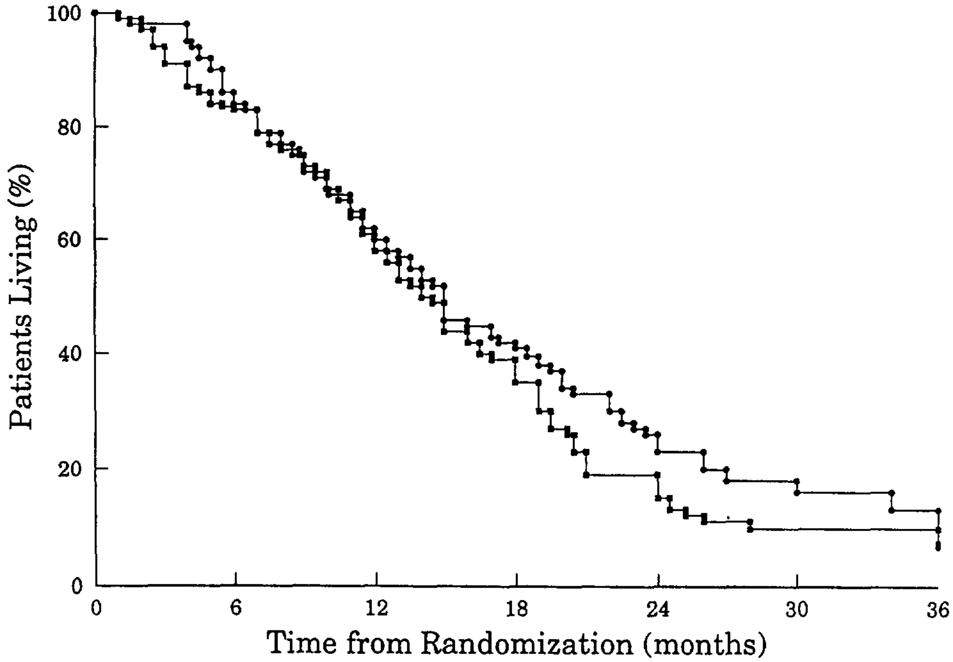

Fig 1. Overall survival for FU/*l*-LV (●---●; n = 119) and FU/racemic LV (■---■; n = 121).

Time to Progression and Survival

At the time of this analysis, less than 2% of the patients are alive without progression. The median time to progression was 6.25 months (95% confidence interval [CI], 5.0 to 8.0) in the FU/racemic LV arm, and 8.0 months (95% CI, 7.0 to 9.0) in the FU/*l*-LV arm, which is of borderline significance (P = .0505).

One hundred eighty-four patients (76.3%) have died. The median follow-up duration for patients still alive is 23 months (range, 17 to 48). Five patients were lost to follow-up for evaluation after 5.5, 7, and 29 months (FU/racemic LV) and after 5.5 and 32 months (FU/*l*-LV), respectively. The median survival time (Fig 1) was not significantly different at 14.5 months (95% CI, 12.0 to 17.0) for patients who received FU/racemic LV versus 15.0 months (95% CI, 12.5 to 18.5) for those who received FU/*l*-LV (P = .28). The 1-year survival rates were 58.3% versus 60.6% (P = .72), and the probability of survival at 2 years was 15.3% versus 23% (P = .16), respectively.

Delivery of Chemotherapy and Toxicity

All eligible patients commenced treatment at full dosage, and 159 (63%; 76 in the FU/racemic LV arm and 83 in the FU/*l*-LV arm) completed the intended 6 months of treatment. Chemotherapy was discontinued prematurely in 82 patients (34%), and there was no significant difference between the two arms in the number who did so (P = .28). The reason for cessation was related to toxicity in 12 patients (15%), including seven in the FU/racemic LV arm and five in the FU/*l*-LV arm (P = 1.0), and because of negative compliance (12 *v* 14) or disease progression in the remainder (27 *v* 17). The percentage of the intended starting dose of FU delivered was only marginally higher in the FU/*l*-LV arm (84% *v* 77%; P = .08). In the FU/racemic LV arm, 22 patients had a dose reduction because of toxicity compared with 16 patients in the FU/*l*-LV arm (P = .38).

Eighty-two (67%) of the 122 patients on study treated with FU/racemic LV reported at least one adverse experience, and 22 (18%) had at least one severe adverse experience. The respective values in the FU/*l*-LV arm were 71 of 119 (60%) and 15 (13%), suggesting no difference between the two arms (P = .23 and P = .29). Hematologic toxicity was commonly noted in both treatment groups (Table 3); leukopenia occurred in 33% (2% grade 3 to 4), granulocytopenia in 30% (4% severe), and anemia in 27%. The median nadir granulocyte counts were 3,323/μL (range, 0 to 12,600) and 3,656/μL (range, 552 to 10,850), and the median nadir platelet counts were 209,000/μL (range, 9,000 to 657,000) and 236,000/μL (range, 69,000 to 745,000) in the FU/racemic LV and FU/*l*-LV arm, respectively. Commonly encountered nonhematologic adverse reactions included nausea/vomiting

Table 3. Treatment-Associated Side Effects

	% FU/LV (n = 122)				% FU/*l*-LV (n = 119)			
	Grade				Grade			
Toxicity	1	2	3	4	1	2	3	4
Leukopenia	23	13	3	2	18	7	—	—
Granulocytopenia	15	16	4	4	14	5	2	—
Thrombocytopenia	6	2	—	—	4	1	—	—
Anemia	27	4	—	—	17	6	—	—
Infection	11	5	—	—	8	3	1	—
Nausea/emesis	17	16	1	—	19	14	3	—
Stomatitis	11	7	6	—	10	7	6	—
Diarrhea	13	10	9	1	10	13	6	1
Alopecia	12	3	—	—	6	4	—	—
Skin	4	1	—	—	2	—	—	—
Peripheral neuropathy	2	—	—	—	2	—	—	—

in 35% (2% grade 3), diarrhea in 32% (7% ≥ grade 3), mucositis in 23% (5% grade 3), and alopecia in 12%. There was significantly greater toxicity experienced by patients in the FU/racemic LV arm only in terms of leukopenia ($P = .01$) and granulocytopenia ($P = .003$). These hematologic side effects were also significantly more likely to be of grade 3 or 4 ($P = .03$). All other differences were not significant at the 5% level. There was only one treatment-related death in a patient who received FU/racemic LV.

DISCUSSION

Numerous phase II and phase III studies of FU given in combination with leucovorin for treatment of advanced colorectal cancer have been conducted.[4,25] These trials, in which the doses of both compounds and their schedules and modalities of administration were varied, produced objective response rates ranging from 15% to 54%. Although randomized trials have established with reasonable certainity that FU/LV-based chemotherapy produces substantial therapeutic gain compared with best supportive care[2,3] or FU alone,[4] until now, it has not been possible to define the regimen that yields optimal antitumor activity. Among several different approaches to refine treatment strategies of fluoropyrimidine biochemical modulation, of current interest seems the levorotatory stereoisomer of leucovorin, presumed to be the active form. Pharmacokinetic and in vitro studies of *l*-LV have shown similar effects at half doses,[15] and improved tumor tissue uptake compared with racemic LV.[14] Encouraging therapeutic results have been reported in recent phase I/II trials in colorectal cancer[16,26] and other malignancies[27,28]; however, any superiority of *l*-LV compared with racemic leucovorin has not been demonstrated yet in a randomized trial.

Together with a preliminary report from the North Central Cancer Treatment Group evaluating equipotent doses of racemic and *l*-LV,[29] this is the first study providing comparative data on the efficacy and toxicity of these two different clinical formulations of leucovorin in combination with FU for the palliative treatment of patients with advanced colorectal cancer. Our results suggest that there are no significant differences between the two regimens in various measures of therapeutic effect, including overall patient survival, progression-free survival, and objective tumor response rate. Except for minor myeloid toxic effects associated with FU plus the levorotatory isomer of leucovorin, there was also no significant difference in terms of treatment-associated side effects. The lower incidence of (severe) leukopenia/granulocytopenia, which had previously been noted in phase I/II studies of FU/*l*-LV[16,28,30] is not clearly understood. Because of the mere absence of cytopenia-related complications in the present study and its rare occurrence in patients treated with FU/racemic leucovorin, this difference might be only of limited clinical significance. It seems noteworthy that in both treatment arms, gastrointestinal symptoms, specifically mucositis and diarrhea, were less frequent and less severe compared with other trials using various dose schedules of FU and racemic LV. The incidence of treatment-associated severe diarrhea and stomatitis reported in the literature ranges up to 23% and 30%, respectively,[31,32] as compared with 8% and 6% in the present series. This phenomenon is most likely to be related to the greater duration of the daily infusions of FU[16,20] used in both arms in the present study, and should be taken into consideration when treating patients with FU/LV to minimize associated toxicity.

In conclusion, this study demonstrated that biochemical modulation of FU by either racemic LV or *l*-LV, the pure active isomer, produced comparable efficacy results in patients with measurable advanced colorectal cancer. Although we cannot exclude that the lack of difference may be attributed to a less-than-optimal dosing of *l*-LV,[33] perfecting the inhibition of thymidylate synthase, in fact, may not be the future to improve therapeutic results in this common malignant disease, neither with further or potentially more effective modulation of FU nor with specific TS inhibitors.[34] Cellular targets, in addition to thymidylate synthase inhibition, will need to be considered to make significant progress in the treatment of advanced colorectal cancer.

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